REMARKS

The Amendments

Claim 1 is amended to recite dysplastic lesion; support for the amendment can be found, for example, at page 2, lines 15-23. Claim 1 is also amended to recite a uterine cervix sample; support for the amendment can be found, for example, at page 8, line 23. Claim 1 is also amended to recite wherein the high risk HPV gene-product is a polypeptide in view of the restriction requirement. Claim 1 is further amended to recite that the presence of cells overexpressing $p16^{INK4a}$ alone is indicative of metaplasias; support for the amendment can be found, for example, at page 10, lines 5-15 and page 11, line 23 through page 12, line 4.

Claim 24 is amended to clarify the meaning of the claim.

New Claim 27 is supported by Examples 1 and 2.

All the other amendments only correct grammatical errors and antecedent basis.

No new matter is added in any of the amendments. The Examiner is requested to enter the amendment and reconsider the application.

The Response

35 USC §112 Second Paragraph Rejections

5. Claims 1-3, 6-17 and 22-24 are rejected under 35 U.S.C. §112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

a. Claim 2 is amended to delete "predominantly."

b. Claim 1 is amended to recite how to discriminate $p16^{INK4a}$ overexpressing metaplasias from $p16^{INK4a}$ overexpressing neoplastic or dysplastic lesions by the wherein clause that the simultaneous presence of cells expressing the high risk HPV gene-product and cells overexpressing $p16^{INK4a}$ is indicative of neoplastic or dysplastic lesion, and the presence of cells overexpressing $p16^{INK4a}$ alone is indicative of metaplasias.

c. The Examiner states that there was no definition of "metaplasias" in the specification. Applicants respectfully submit that the term was known to a person skilled in the art at the

time of filing the application. For example, the term "metaplasia" is defined in "Atlas of Tumor Pathology - Tumors of the Cervix, Vagina, and Vulva by R. Kurman, H. Norris and W. Wilkinson; published by the Armed Forces Institute of Pathology, Washington D.C. 1992. On page 11, right column, starting with the second paragraph, the term metaplasia is explained. (Copy attached) Further, the application describes, "Metaplastic cells give rise to a patchy or focal staining pattern, whereas neoplastic lesions give rise to diffuse staining pattern. Moreover, the staining intensities of metaplastic cells are predominantly less than that of neoplastic cells." (page 1, lines 21-25)

The Examiner also states that the definitions of "metaplasias" and "preneoplastic" are overlapping. Applicants do not agree with the Examiner. However, to further prosecution, Applicants have deleted "preneoplastic" and inserted "dysplastic." Examples 1 and 2 show the discrimination of metaplasia and dysplasia by the present method.

Therefore, the §112 second paragraph rejections should be withdrawn.

7. Claims 1-3, 6-7, 9, 12-17 and 22-24 are rejected under 35 U.S.C. §112, first paragraph, because the specification does not reasonably provide enablement for a claimed method using any biological sample.

Applicants have amended Claim 1 to recite a uterine cervix sample as the Examiner has suggested.

Therefore, the §112 first paragraph rejections should be withdrawn.

35 USC §103(a) Rejections

9. Claims 1-3, 6-17 and 22-24 are rejected under 35 U.S.C. §103(a) as allegedly being unpatentable over Klaes et al. (2001, Int. J. Cancer 92:276-284) in view of Solomon et al. (2001, J. of the National Cancer Institute 93(4):293-299) and Guccione (Virology 293:20-25 (2002)), as evidenced by von Knebel Doeberitz (2001, Dis. Markers 17(3):123-8 (abstract only). The rejection is traversed.

The Present Invention

Metaplasia may be easily discriminated from dysplasia or neoplasia in histology by the location in the tissue. However, in cytology, the tissue information does not exist. The

present invention identifies the problem that about 30% of metaplastic cells show some immunoreactivity with $p16^{INK4a}$ specific antibodies, and thus are stained in the course of the cytological testing procedures (page 1, lines 20-21). **This is a unique problem not recognized by any of the cited prior art.**

The present invention provides a method for discriminating $p16^{INK4a}$ overexpressing metaplasias from $p16^{INK4a}$ overexpressing neoplastic or dysplastic lesions in a uterine cervix sample in the course of cytological testing procedures. The discrimination method is based on the presence or absence of (a) cells expressing a high risk HPV gene-product and (b) cells overexpressing $p16^{INK4a}$ in the same uterine cervix sample.

Klaes et al.

Klaes et al. do not recognize the problem that about 30% of metaplastic cells show some immunoreactivity with $p16^{INK4a}$ specific antibodies and that measuring $p16^{INK4a}$ alone cannot discriminate metaplasia from neoplasia.

Klaes et al. disclose that Pap test relies on subjective diagnostic parameters and has high rate of false-positive and false-negative results, therefore, objective diagnostic parameters are desirable. Klaes et al. teach that $p16^{INK4a}$ staining, if applied in histology and cytology applications, may function to improve the assessment of diagnosis compared to conventional staining such as H&E staining in Histology or PAP staining in Cytology.

Klaes et al. report that $p16^{INK4a}$ is a **specific** biomarker to identify dysplastic and neoplastic cervical epithelia in sections of cervical biopsy samples or cervical smears. (see last sentence of Abstract and Title). Klaes et al. do not describe any problems with the p16 staining when used in cytology.

Accordingly, Klaes et al. teaches that $p16^{INK4a}$ alone is a sufficient biomarker and no need for combination with any further marker molecule. Based on Klaes et al., a person of ordinary skill in the art would not be motivated to seek for a further marker to perform the method of discriminating $p16^{INK4a}$ overexpressing metaplasias from $p16^{INK4a}$ overexpressing dysplasias and neoplasias because Klaes et al. teaches that $p16^{INK4a}$ is a marker that sufficiently specific for identification of dysplastic cervical cells.

Solomon et al.

Solomon et al. disclose testing cancer-associated HPV DNA in solubilized cell samples, in women referred to colposcopy. Solomon teaches that sensitivity in detection of high grade lesions may be increased by adding hc2 to cytology testing. One must note that cytology and repeated cytology in Solomon refer to PAP staining and not to determine the protein levels of a biochemical marker. Solomon et al. conclude that hc2 testing for cancer-associated HPV DNA is a viable option in the management of women with ASCUS. It has greater sensitivity to detect CIN3 or above and specificity comparable to a single additional cytologic test indicating ASCUS or above. (see Abstract) There is no hint in Solomon et al. that the HPV DNA testing should be combined with another testing of a different biochemical marker.

Solomon et al. describe that "Additional analyses will examine whether the specificity of HPV testing can be improved without sacrificing sensitivity by raising the threshold for a positive result, by tailoring recommendations based on patient age, or be lengthening the time interval from index ASCUS cytology result to HPV testing." (page 298, right column, last paragraph) **Although Solomon et al. suggest different ways of improving the HPV testing; there was no mentioning in Solomon et al. as to combining a different biochemical marker, particularly $p16^{INK4a}$, to improve the test result.**

As acknowledged by the Examiner, Solomon et al. do not teach or suggest the detection of $p16^{INK4a}$ at all.

Further, Solomon et al. tested HPV DNA by solubilizing the cells, Solomon et al did not test HPV protein or use a cytological testing procedure (Claim 1).

Guccione et al.

First of all, Guccione et al. do not mention $p16^{INK4a}$ at all. Guccione et al. do not cure the deficiency of Klaes et al. and Solomon et al.

Further, Guccione only teaches detection of recombinant HPV E7 protein that have been genetically modified to bear a haemagglutinin tag for enhanced detection. (HA-tagged HPV-11E7 vectors were used; see page 24 right column under "Materials and Methods") Guccione et al do NOT (a) detect native levels of E7 but only detect the levels that may arise due to transfection with expression vectors, and (b) detect HPV proteins per se but only detect

tagged proteins. Accordingly, the information given in Guccione cannot be transferred to the detection of HPV proteins in native cells because (a) the protein levels are not comparable in native cells and in transfected cells (the levels in transfected cells are much higher and thus easier to detect in immunochemical methods), and (b) the detection of Guccione is based on antibodies directed to haemaglutinin tag and not directed to the HPV expression product. Such indirect approach for detection is not viable in native cells and does not make obvious the method of detection of HPV proteins directly in native cells (Claim 1).

Combination of References

Solomon and Klaes both teach methods for improving conventional cervical cancer screening programs by using a biochemical marker test to increase sensitivity. However, they do not disclose using the biochemical marker test to improve specificity.

Klaes et al. identify $p16^{INK4a}$ as a specific biomarker. Klaes et al. do not recognize the false-positive problem of $p16^{INK4a}$ in metaplastic cells. Reading Klaes et al., a person skilled in the art would have no reason to combine another biochemical marker with $p16^{INK4a}$ to improve the specificity of the test, which discriminates metaplasia from dysplasia and neoplasia.

The Examiner states that it would be obvious for a skilled person to substitute the immunohistochemical detection of $p16^{INK4a}$, as taught by Klaes, for the cytological component of the cytological/HPV detection combination method taught by Solomon to discriminate $p16^{INK4a}$ overexpressing metaplasias from $p16^{INK4a}$ overexpressing neoplastic lesion, because of the advantages taught by Klaes in the use of objective diagnostic parameters in avoiding the false negatives and false positives of the subjective Pap test. The Examiner's statements are purely hindsight and incorrect.

First, based on the advantages taught by Klaes regarding the $p16^{INK4a}$ testing, there is no motivation to add an additional HPV testing. Second, the HPV testing of Solomon was performed on solubilized cells (non-cell based testing procedure) and was not performed in the course of a cytologic testing procedure. Therefore, even the hindsight combination of Klaes and Solomon does not produce the claimed invention.

Applicants have discovered that two different biochemical markers of $p16^{INK4a}$ and the high risk HPV gene-product are complementary for discriminating metaplasias from neoplastic or dysplastic lesions. Applicants have reduced the invention to practice (see Examples 1 and

2). **The cited references neither discovered the problem nor provided a solution to the problem.**

For the reasons stated above, the Examiner is requested to withdraw the 103(a) rejection of Claims 1-3, 6-8, 11-17 and 22-24.

CONCLUSION

Applicants believe that the application is now in good and proper condition for allowance. Early notification of allowance is earnestly solicited.

Respectfully submitted,

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Enclosure: Tumors of the Cervix, vagina, and Vulva, pages 10 and 11

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